## II. RESEARCH PROTOCOL

### A. Specific Aims

Squamous cell carcinoma (SCCa) constitutes the vast majority of head and neck cancers. If identified early, these cancers can be treated relatively easily either surgically or radiotherapeutically with an excellent cure rate. Unfortunately most cancers are diagnosed relatively late with the cancer at an advanced state. The standard form of treatment for these patients with advanced head and neck SCCa is surgical resection followed by radiation therapy but with a five year survival of under 50%. In addition, there is a significant subset of patients who are so advanced at the time of presentation that they are regarded as incurable by conventional therapy including chemotherapy which has yielded little change in survival of these patients. Likewise, patients who recur after conventional therapy have a dismal prognosis. Of equal concern is that the quality of life for these patients is extremely poor with local disease interfering with vital functions e.g. swallowing, breathing, etc.

This study aims to utilize a new form of treatment for advanced head and neck cancer in an attempt to stimulate the patients own immune system to attack the cancer. This will be accomplished by injecting the gene HLA-B7 into the tumor to increase the tumors' expression of major histocompatibility complexes. This approach has shown promise in other types of malignancy, and it is believed that head and neck cancer presenting at an advanced stage or with recurrence would be an ideal model to study as the cure rate is extremely poor and the tumors are easily monitored. It is hoped that this approach may cause sufficient local control that patient morbidity would be decreased and perhaps even patient survival prolonged.

This approach may be extended from the upper aerodigestive tract to also include obstructing tumors of the trachea and mainstem bronchi. As with the head and neck tumors described above, these tumors are not curable by conventional therapy and can be directly accessed for intratumoral injection. The bronchial obstruction significantly impacts quality of life, and this gene therapy approach may be cytoreductive and result in decreased patient morbidity and prolonged survival.

### B. Significance

Cancer is a disease in which certain cells grow uncontrolled by the body's normal self-regulatory mechanisms. Traditional chemotherapy seeks to control cancer by killing rapidly dividing cells or by preventing cells from entering the cell cycle and dividing. However, rapidly dividing non-malignant cells such as bone marrow and intestinal epithelium are highly susceptible to the toxicity of chemotherapy. Doses sufficient to induce remission in the cancer cannot be administered without life-threatening side effects in 5- 10% of the patients and an overall mortality of 0.5%. In addition, many solid malignancies have relatively few cells in division at any one time and have long doubling times. A therapeutic approach that selectively kills tumor cells with high selectivity and efficacy would theoretically be far superior to currently available therapies.

The goal of immunotherapy is to stimulate the immune system to recognize and kill cancer cells by modifying the tumor cells or modifying the host response by such mechanisms as expanding the population of lymphocytes that respond specifically to the antigens on the tumor cells. Immunotherapy has shown promise as an approach to the treatment of malignancy. Indeed, cancers such as melanoma, renal cell carcinoma and colon adenocarcinoma are responsive to modulation of

immune function, because the immune system can be induced to recognize tumor-associated and tumor-specific antigens in these cells.

Over the last several decades, there have been many attempts to identify tumor-specific antigens that might be the targets for cytotoxic antibodies or cell-mediated immunity. There have been numerous attempts to develop vaccines and monoclonal antibodies directed at one or more preferentially expressed cell surface antigens in a variety of cancers. Overall, tumor vaccines using intact cells or extracts plus adjuvants have resulted in approximately a 10-20% response rate in patients with metastatic cancers. Other approaches to immunotherapy have involved the administration of non-specific immunomodulating agents such as Bacillus Calmette-Guerin (BCG), cytokines such as IL-2 or IFN-α, and/or adoptive transfer cytotoxic T cells, which have shown promise in animal models (1-6) and in man (7-10). More recently, molecular genetic interventions have been designed in an attempt to improve the efficacy of immunotherapy.

Dr. Gary Nabel and colleagues at the University of Michigan laid the groundwork for the molecular genetic approach that enhances the immune response to tumors by *in vivo* gene transfer. This immunotherapeutic approach based on animal model work ( 11, 12) uses a gene encoding a transplantation antigen, an allogeneic class I major histocompatibility complex (MHC) antigen, HLA-B7. This gene is introduced into human tumors *in vivo* by direct injection of plasmid DNA that expresses the HLA-B7 on the surface of the tumor cells. Expression of allogeneic MHC antigens on tumor cells stimulates immunity against both the transfected cells as well as previously unrecognized antigens present in unmodified tumor cells (13). The introduction of an allogeneic MHC gene directly into tumor *in* vivo has induced partial tumor regressions, as well as specific cytotoxic T cell responses to other antigens ( 13).

This approach was based on the belief that a deficient expression of class I MHC molecules limits the ability of tumor cells to present antigens to cytotoxic T cells. Freshly isolated cells from naturally occurring tumors frequently lack class I MHC antigen completely or show decreased expression (14-18). Reduced class I MHC expression can also facilitate growth of these tumors when transplanted into syngeneic recipients. Several tumor cell lines which exhibit low levels of class I MHC proteins become less oncogenic when expression vectors including the relevant class I MHC antigens are introduced (19-23). In some experiments, tumor cells which express a class I MHC gene confirm unity in naive recipients against the parental tumor (20,21).

In a preliminary trial with 5 malignant melanoma patients, Nabel has demonstrated: 1) evidence of gene transfer on biopsy of the injected tumor by measuring plasmid mRNA and cell surface expression, 2) an immune response in 2 patients where cell lines were established from the tumor and lysing of autologous tumor cells occurred 3) a partial remission which involved cutaneous and visceral disease and 4) no adverse effects from injecting plasmid DNA (complexed with a cationic/neutral lipid combination) into human melanoma (13). These data suggest that tumor cells modified with the HLA-B7 gene not only stimulate CTLs and potentially other immune system cells to recognize tumors expressing HLA-B7, but they may also provide a stimulus to immune cells to eliminate tumor cells at other sites which express tumor-associated antigens in association with the patient's own HLA antigens. The discovery that HLA-B7, when expressed on the surface of melanoma tumor cells, stimulates an immune response to both the transfected antigen and other tumor antigens inspired the systematic development of Allovectin-7 as a candidate for cancer

immunotherapy. Allovectin-7 is the product name. VCL 1005 is the code name for the specific DNA plasmid construct. The plasmid is formulated with a cationic/neutral lipid mixture (DMRIE/DOPE), which complexes with the DNA.

In this study Allovectin-7 will be injected directly into the tumor. The HLA-B7 will be able to enter the tumor cells due to the lipid mixture which fuses with the cell membrane and deposits the DNA within the cell. The plasmid DNA that is in complex with the cationic lipid is non-replicating and is composed of the DNA for the HLA-B7 gene. The cells immediately surrounding the injection site will be able to be transfected, and thereby express HLA-B7. The cells that then express HLA-B7 will be recognized by the immune system as foreign and rejected. The cells that are transfected will be mostly tumor cells since the injection will be directly into the tumor, but a small number of the surrounding cells may be affected. This should not cause significant cytotoxicity, and would be far less noxious to normal tissue than any conventional therapies (i.e. surgery or radiation) which have significant affects on the surrounding normal tissue. Health care providers will not be at risk from Allovectin-7 since it needs to be injected to transfect tissue and only contains non-replicating DNA.

Given the promising results obtained in the melanoma patients we wish to evaluate this technology for head and neck SCCa which has proved refractory to conventional therapy. We believe head and neck SCCa to be an ideal tumor to study because these tumors are highly aggressive in behavior, are refractory to conventional therapy, and are readily available for monitoring by clinical evaluation, imaging and are easily biopsied. While this study would initially be conducted in advanced tumors refractory to conventional therapy, it is hoped that this approach would become a part of the armamentarium for the management of early cancers.

No curative therapy currently exists for obstructing non-small cell lung cancers of the respiratory tract, and like the head and neck cancers described above, these tumors are accessible for injection, although bronchoscopy would be required. Others have bronchoscopically injected lung tumors with other methods of gene therapy, attempting to restore the function of the p53 tumor suppressor gene (24). These injections resulted in p53 gene expression, however, that method of gene therapy failed to produce lasting disease remission. Given our promising results obtained in treating advanced head and neck cancer patients, we propose further extending the indications for HLA-B7 based gene therapy to include not only the upper aerodigestive tract, but also the trachea and mainstem bronchi. Treatment would be combined with broncho-alveolar lavage (BAL) to more effectively monitor the immune response to this immune modulating gene therapy.